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Butterfly wing colors: glass scales of *Graphium sarpedon* cause polarized iridescence and enhance blue/green pigment coloration of the wing membrane

Doekele G. Stavenga[1,*], Marco A. Giraldo[1,2] and Hein L. Leertouwer[1]

[1]Department of Neurobiophysics, University of Groningen, Physics-Chemistry Building, Nijenborgh 4, Groningen, 9747 AG, The Netherlands and [2]Institute of Physics, University of Antioquia, Medellín, AA 1226, Colombia

*Author for correspondence (d.g.stavenga@rug.nl)

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SUMMARY

The wings of the swordtail butterfly *Graphium sarpedon nipponum* contain the bile pigment sarpedobilin, which causes blue/green colored wing patches. Locally the bile pigment is combined with the strongly blue-absorbing carotenoid lutein, resulting in green wing patches and thus improving camouflage. In the dorsal forewings, the colored patches lack the usual wing scales, but instead have bristles. We have found that on the ventral side most of these patches have very transparent scales that enhance, by reflection, the wing coloration when illuminated from the dorsal side. These glass scales furthermore create a strongly polarized iridescence when illuminated by obliquely incident light from the ventral side, presumably for intraspecific signaling. A few ventral forewing patches have diffusely scattering, white scales that also enhance the blue/green wing coloration when observed from the dorsal side.

Key words: imaging scatterometry, sarpedobilin, bile pigments, lutein.

INTRODUCTION

Graphium is a genus of swallowtail butterflies, known as swordtails or kite swallowtails, from Australasian and Oriental regions. A widespread species is the Common Bluebottle *Graphium sarpedon* (also called Blue Triangle). Its wings are marked by bands of blue/green patches contrasted by brown/black borders. *Graphium sarpedon* has the rare property that the blue/green wing coloration is created by pigments in cells constituting the wing membrane (Allyn et al., 1982; Nijhout, 1991), in contrast with virtually all butterfly species where the wing coloration is due to the tapestry of scales that covers the wings. The colors of butterfly scales result from pigments that selectively absorb in certain wavelength ranges and are embedded in the scale structures, which reflect and scatter light that has not been absorbed by the pigments. Alternatively, or in addition to this pigmentary coloration, wave-optical interference phenomena cause so-called structural coloration (Berthier, 2003; Kinoshita et al., 2008; Kinoshita, 2008).

The pigments coloring the wings of *G. sarpedon* were identified to be the bile pigment sarpedobilin (Choussy et al., 1973; Barbier, 1981) and the carotenoid lutein (Allyn et al., 1982; Rothschild and Mummery, 1985). Together they create a green color that resembles the color of leaves and thus the wing pigmentation is assumed to have a function in camouflage (Rüdiger, 1970; Kayser, 1985). Green colored wings can be found in many butterfly species, but the optical mechanisms involved are amazingly diverse. For example, the broad-band green wings of the lycaenid *Chrysozephyrus aurorinus* have scales where the lumen consists of a stack of chitin layers that functions as a multilayer reflector (Wilts et al., 2009). The wings of the Green Hairstreak, *Callophrys rubi* have ventrally scales filled with chitin structures shaped into gyroids, which function as photonic crystals (Michielsen and Stavenga, 2008; Michielsen et al., 2010). The gyroids form domains, which scatter directionally blue and yellow light but because the domains are oriented randomly, a matte green color of the scales results; a similar scale organization is found in a related lycaenid, *Cyanophrys remus* (Kertesz et al., 2006). The optical method of creating color by mixing various proportions of different colors of light, called additive color synthesis, is also applied by the papilionid *Papilio palinurus*, although in a completely different way (Vukusic et al., 2001). Here the scales form sculpted multilayers acting as polarizing retro-reflectors for both blue and yellow light. The Madagascan sunset moth, *Chrysiridia rhipheus*, similarly practices polarization-sensitive color mixing, but with highly curved wing scales that partially overlap (Yoshioka and Kinoshita, 2007).

The pigmented wing patches of *G. sarpedon* have scales and/or bristles (Ghiradella, 1998) that will also contribute to the wing coloration, but how the interplay of wing pigmentation and scale and bristle ornamentation contributes to the blue/green coloration of *G. sarpedon* has not been investigated into any detail. In the present study we will particularly focus on the role of the glass scales on the ventral side of the wings, which by reflection enhance the blue/green pigment wing color and exhibit a distinct polarized iridescence when illuminated obliquely.

MATERIALS AND METHODS

Animals

The optical and structural measurements were performed on the Japanese subspecies *Graphium sarpedon nipponum*. Specimens were captured around the Sokendai Hayama campus, Kanagawa, Japan, and further obtained with the help of Prof. K. Arikawa and students. Additional pinned *G. sarpedon* specimens were purchased from Robert Goodden, Worldwide Butterflies, Sherborne, Dorset, UK, one of which was photographed as before [Fig. 1; for methods see Wijnen et al. (Wijnen et al., 2007)]. Specimens in the collection of the National Museum of Natural History, Naturalis (Leiden, the Netherlands) were also investigated.

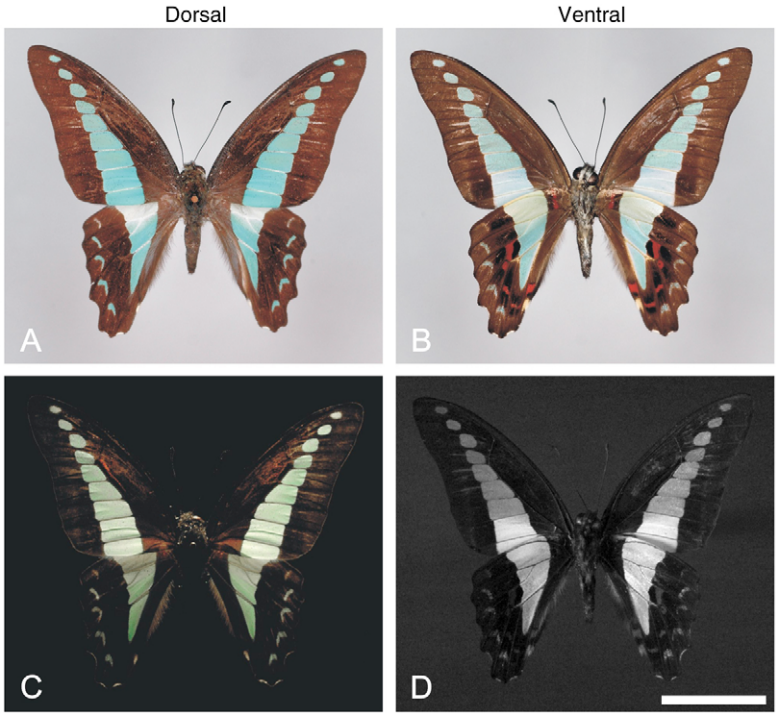

Fig. 1. The swordtail butterfly *Graphium sarpedon* photographed with white-light epi-illumination in dorsal (A) and in ventral view (B), with white-light trans-illumination in dorsal view (C) and with ultraviolet-light epi-illumination in ventral view (D; bar: 2 cm).

Microscopy

The photographs of the wing patches (Figs 2–4) were made with an Olympus SZX16 stereomicroscope and an Olympus DP70 digital camera (Tokyo, Japan). The side-view of a single glass scale (Fig. 5A) was also made with this instrument, but the normal view (Fig. 5B) was made with a Zeiss Universal microscope, using a ×40 Epiplan objective (Oberkochen, Germany). Scanning electron microscopy (SEM) was performed with a Philips XL-30 ESEM (Eindhoven, The Netherlands).

Spectrophotometry

Reflectance spectra of wing patches were measured with a bifurcated, flexible fiber-optic probe connected to a CCD detector array spectrometer (AvaSpec-2048, Avantes, Eerbeek, The Netherlands), using a deuterium/halogen light source [AvaLight-D(H)-S]. A white diffuse reference tile (Avantes WS-2) served as reference. The reflectance spectra from more or less specular surfaces are therefore only reliable in relative terms.

For angle-dependent reflectance measurements (ARM) of wing patches one end of an optical fiber was connected to the deuterium/halogen light source and the other end was mounted at a goniometer together with a small lens, which focused the fiber tip at the goniometer's rotation axis. The tip of a second fiber was mounted with a similarly focusing lens at a second goniometer and its other end was connected to the spectrometer. The rotation axes of the two goniometers coincided and the two fibers rotated in the same plane. The patch to be measured was positioned in this plane and at the axis of rotation of the goniometers.

Reflectance and transmittance spectra of the wing membrane were acquired with a microspectrophotometer (MSP), consisting of a xenon light source, a Leitz Ortholux microscope and an S2000 fiber optic spectrometer (Ocean Optics, Dunedin, FL, USA). The microscope objective was an Olympus ×20, NA 0.46 (Olympus). The absorption by the pigments in the wing membrane was also measured with an experimental setup consisting of two aligned optical fibers; one fiber delivered the light and the second one captured the transmitted light and relayed it to the AvaSpec-2048 spectrometer (Avantes).

Imaging scatterometry

The spatial distribution of the light scattered by the wing was visualized with an imaging scatterometer (ISM) (Stavenga et al., 2009) (see also Vukusic and Stavenga, 2009). A small wing piece was therefore glued to a glass micropipette, the slender tip of which was pulled with a P-97 Flaming/Brown micropipette puller (Sutter Instrument, Novato, CA, USA). The wing piece thus could be positioned in the first focal plane of an elliptical mirror, where it was viewed and photographed *via* a small, axial hole in the mirror. The wing piece was illuminated from various angles *via* a half mirror. The light scattered by the wing over a hemisphere was collected, *via* a diaphragm in the elliptical mirror's second focal plane, with a lens and subsequently imaged onto a digital camera (Olympus DP70). The images, corrected for the optical distortions of the setup, are presented as polar plots (for details, see Stavenga et al., 2009).

RESULTS

Both the forewings and the hindwings, dorsally as well as ventrally, of the Common Bluebottle, *Graphium sarpedon*, are marked by a central band of blue/green patches surrounded by brown/black borders (Fig. 1). On the forewings nine patches can be discriminated, which we have numbered Df1–9 (Df, dorsal forewing), when observed from the dorsal side (Fig. 1A), and Vf1–9 (Vf, ventral forewing), when observed from the ventral side (Fig. 1B), with Df1 and Vf1 nearest to the hindwing and Df9 and Vf9 near the wing tip. The hindwings have three distinct colored patches, a number of blue/green colored lunules and ventrally a few small red areas. The blue/green patches are quite transparent in the blue/green wavelength range, which is apparent when observing the wings from the dorsal side while the illumination is from the ventral side

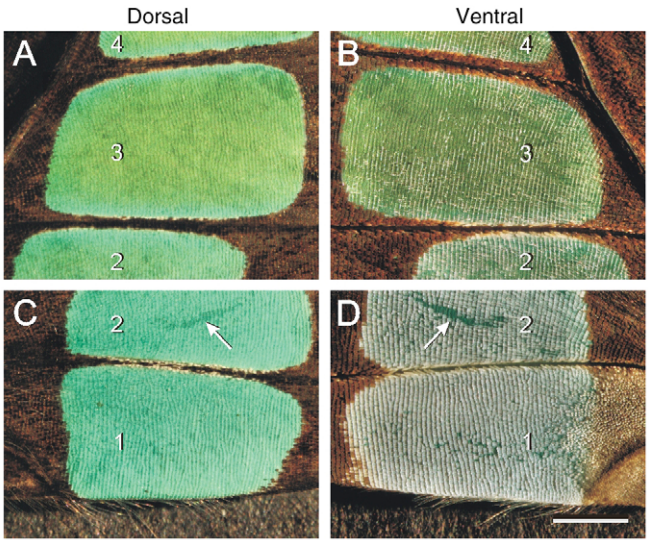

Fig. 2. Photographs of the dorsal (A,C) and ventral (B,D) forewing of a male *Graphium sarpedon nipponum* of the colored wing patches numbered 1–4, with number 1 nearest to the hindwing. Patches 1 and 2, and the rim of patches 3 and 4, are dorsally blue/green. The latter patches are mainly green. The wing membrane of patches 1 and 2 is blue/green (C), but the patches are ventrally white because of diffusely scattering white scales (D); the blue/green color of the wing emerges where the scales are lacking (C,D, arrows; bar: 2 mm).

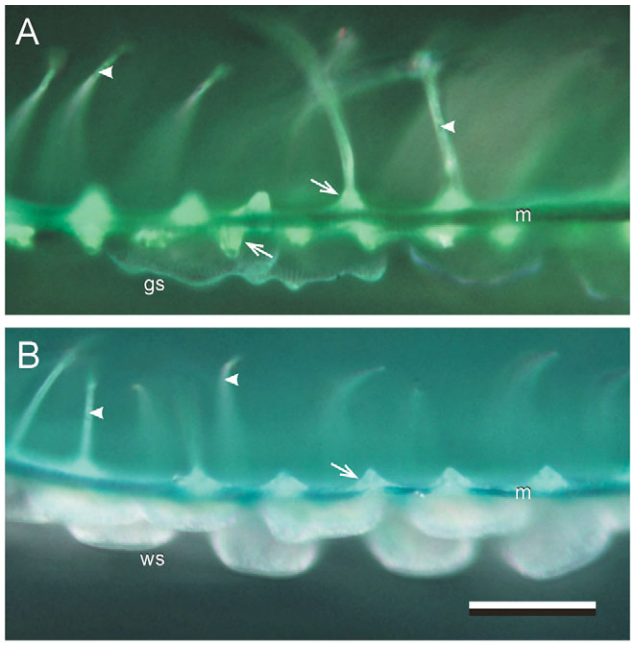

Fig. 3. Side views of patches number 3 (A) and number 1 (B) of a sectioned forewing of a male *Graphium sarpedon nipponum*. The sockets (arrows) stand out of the wing membrane (m). Both patches have long bristles (arrow heads) on the dorsal side, but patch 3 has on the ventral side glass scales (gs) and patch 1 has white scales (ws) (bar: 50 μm).

(Fig. 1C). All colored patches moderately reflect ultraviolet light; slightly more on the ventral side of the wings (Fig. 1D) and particularly so in Vf1, Vf2 and Vh1 (Vh, ventral hindwing patch nearest to the forewing), which are whitish colored when observed with visible light.

The hue of the colored patches varies among the *G. sarpedon* subspecies. We have observed the strongest color differences in the Japanese subspecies *G. s. nipponum*, which we used in the measurements described hereafter. A closer look at the forewing patches 1–4 (Fig. 2) shows a strong difference in coloration of the dorsal (Fig. 2A,C) and ventral (Fig. 2B,D) sides, indicating a different structural and/or pigmentary organization of the two sides of the wings. White scales cover the ventral forewing patches Vf1 and Vf2 (Fig. 2D), which is not the case for Vf3, which has a strongly different color (Fig. 2B). The patches Df1 and Df2 are colored light blue/green (Fig. 2C), but a darker color is observed where the white scales at the ventral side are missing due to damage (arrows in Fig. 2C,D). This demonstrates that light incident from dorsally, after passing the wing membrane, is backscattered by the white scales; thus, contributing to the coloration seen from dorsally. The dorsal forewing patch Df3, except for its rim (Fig. 2A), is distinctly greener than Df1 (Fig. 2C), indicating differences in pigmentation.

The organization of the wings in the colored patches can be demonstrated by viewing a sectioned wing from its side (Fig. 3). It appears that the wings in the colored patches dorsally only have bristles, attached to the wing membrane in densely colored sockets. Only ventrally scales exist (together with some bristles), which are colorless in Vf3 (Fig. 3A) and Vf1 (Fig. 3B). The wing membrane, sockets as well as the bristles are green in Df3 and blue/green in Df1.

To further investigate the asymmetry of the two wing sides, we illuminated Df3 and Vf3 with linearly polarized light and observed the patches through a parallel (Fig. 4A,B) and a crossed (Fig. 4C,D) analyzer. It thus appeared again that the patch dorsally had only bristles (Fig. 4C) and ventrally had scales (Fig. 4B). The scales are locally strongly reflecting, but a crossed analyzer extinguishes the reflection and reveals the sockets in regular rows (Fig. 4C,D); thus, demonstrating that the scales are unpigmented and very transparent. We therefore call these ventral scales glass scales. The green color of the patch appears to emerge from mainly the socket cells, the roots of the dorsal bristles and the ventral glass scales (Fig. 4C,D). The other cells that make up the wing membrane also have a green tinge but evidently the pigment density outside the socket cells is lower. As noted above, the wing membrane is in fact quite transparent (Fig. 1C), because the pigmented socket cells on the opposite side of the wing shine clearly through (Fig. 4C,D). Indeed, Df3 illuminated with linearly polarized light incident from dorsally and observed with a parallel analyzer shows a substantial reflection by the ventral glass scales, notwithstanding some filtering by the wing membrane (Fig. 4A compared with 4C). In the opposite case, that is, observing Vf3 with parallel polarizer and analyzer, the reflection of the ventral glass scales is even distinctly larger than that of the wing membrane (Fig. 4B compared with 4D).

Fig. 4B shows that the glass scales are not flat but somewhat wrinkled. The scales hence will not act as simple glass panes, but reflect into somewhat different directions, depending on their shape. To learn more about the shape of the scale, we glued a single glass scale to a micropipette and observed it from sideways (Fig. 5A). The lateral deviations from flatness appear to be rather minor, but the scale is curved upwards in the longitudinal direction near its tip. Observed with a transmitting light microscope (Fig. 5B), the scale appears to have clear ridges, but otherwise the scale is highly transparent.

The reflection properties of butterfly wing scales depend on their fine structure and therefore we have performed SEM (Fig. 6). The hairs or bristles of the dorsal forewing (Fig. 6A), the glass scales

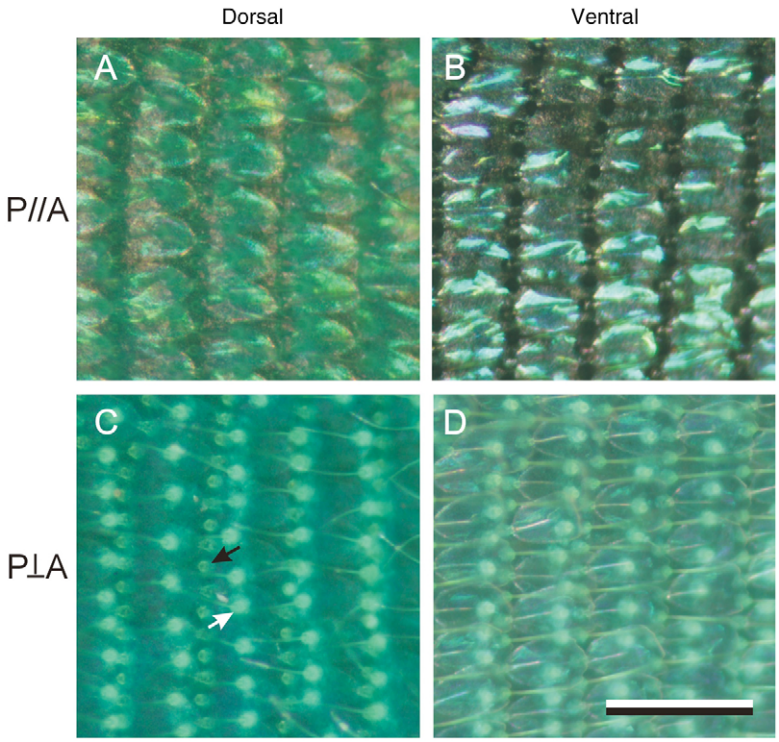

Fig. 4. Photographs of a small area of the dorsal forewing patch Df3 (A,C) and its opposite side, the ventral forewing patch Vf3 (B,D), illuminated with linearly polarized light and observed with a parallel analyzer (A,B) or a crossed analyzer (C,D); exposure times A:B:C:D=1:2:2:5. The reflections of the ventral glass scales shine through the wing in A and are prominently visible in B. They are extinguished in C and D, and then reveal the dorsal bristles and the socket cells; the white and black arrows in C indicate a dorsal and ventral socket, respectively (bar: 200 μm). P is for polarizer, A is for analyzer, which are parallel (//) of perpendicular (crossed, ⊥) to each other.

(Fig. 6B) and the white scales (Fig. 6C) of the ventral forewing are all marked by the longitudinal ridges, with overlapping lamellae, connected by cross ribs. The spacing of the cross ribs is rather similar in the three cases (170±30 μm), illustrating the common origin of the bristles and scales (Ghiradella, 1998). The scales can be considered as flattened bristles, with only small holes in the glass scales and large windows in the white scales. The optical consequences of the anatomical differences are as is already indicated by the names. The thin, hair-like bristles only slightly scatter light and thus contribute little to the wing coloration. The glass scales are unpigmented and transparent and have a virtually continuous upper lamina, with only small holes, so that they also act as reflectors. The white scales have large and very irregularly shaped windows, which result in diffusely scattering structures that cause the matte white color of Vf1 and Vf2 (Fig. 2D).

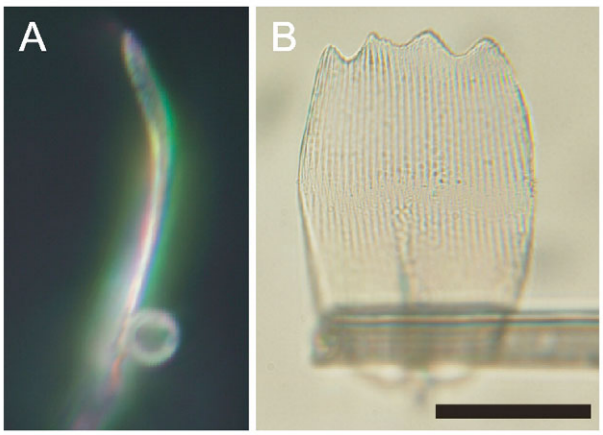

Fig. 5. A single glass scale glued to a glass micropipette in air, with epi-illumination viewed sideways (A) and in normal view with transmitted light (B; bar: 50 μm).

To better understand the differences in the blue and green coloration of the dorsal forewing patches, we have performed MSP measurements. Fig. 7A presents reflectance spectra, $R(\lambda)$, of forewing sockets in the blue/green (Df1) and green (Df3) patches. The absorbance spectra, calculated as $A(\lambda)=-\log_{10}[R(\lambda)/R(\lambda_0)]$, with λ_0=750 nm, demonstrate the presence of a main pigment absorbing maximally at 670 nm, with a shoulder at about 620 nm; the green patches have in addition a pigment with a few absorption maxima in the blue wavelength range (Fig. 7B). Previous work on *G. sarpedon* by Choussy et al. revealed that the wings contain the bile pigment sarpedobilin (Choussy et al., 1973), and, furthermore, Rothschild and Mummery found that the wings in addition contain the carotenoid lutein (Rothschild and Mummery, 1985). For comparison, Fig. 7C presents spectra from the literature of two related bile pigments (Saito, 1998) and lutein (Jouni and Wells, 1996). Additional MSP, on areas in between the sockets, yield reflectance spectra similar to but lower than those of Fig. 7A, showing that the wing membrane cells other than the socket cells in the same patch contain similar pigments, but in lower concentration, in agreement with the visual observations described above. Transmission (micro)spectrophotometry on wings immersed in a fluid matching the refractive index (xylene) (see Stavenga et al., 2004) showed that the absorption of the wing membrane in the far-red (near the 670 nm peak wavelength) and in the UV is around 70±10%, but in the blue/green wavelength range it is no more than 20±5%.

Having detailed the different components of the colored wing patches that scatter and reflect incident light, i.e. the wing membrane and the scales, we subsequently studied how they together determine the color of a wing patch or its reflectance spectrum. Measurements with a fiber-optic probe and a spectrometer (Fig. 8A) show that the dorsal forewing patches Df1 and Df2 have a broad reflectance peak

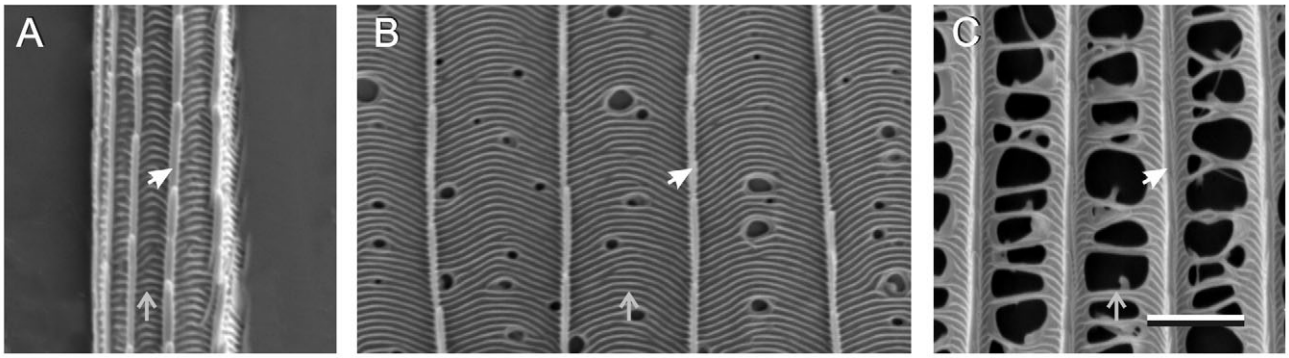

Fig. 6. Scanning electron microscope photographs of a dorsal bristle (A), a ventral glass scale (B) and a ventral white scale (C; bar: 2 μm). The white, oblique arrows indicate the ridges, and the grey, vertical arrows indicate the cross ribs. The glass scale has small holes, but the white scale has large holes, called windows.

around 500 nm, which is distinctly higher than the reflectance of the patches Df3 and Df4. This is partly due to the absorption by lutein in Df3 and Df4. Yet, the peak reflectance of both Df1 and Df2 is enhanced, because light transmitted by the wing membrane in Df1 and Df2 is noticeably backscattered by the white scales of Vf1 and Vf2, on the ventral side of Df1 and Df2, and part of this backscattered light passes the wing membrane at the way back without being absorbed. The mirroring glass scales of Vf3 and Vf4 play a similar role for the reflectance of Df3 and Df4 (see Figs 2 and 3).

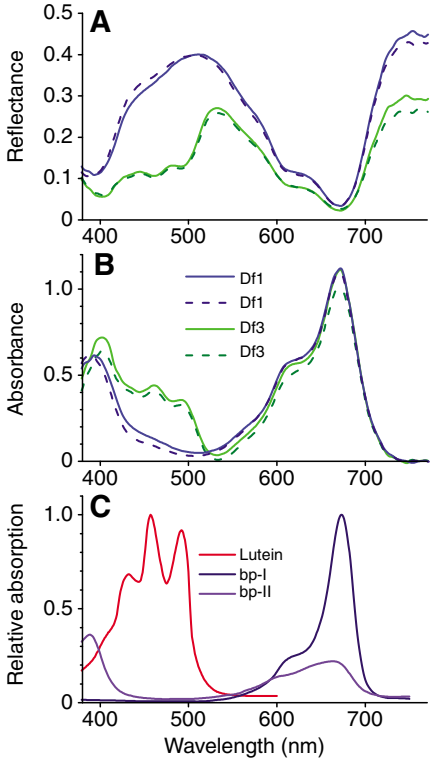

Fig. 7. Reflectance spectra of dorsal sockets, two in Df1 and two in Df3 (A), converted to absorbance spectra (B). The absorption spectra (C) of two bile pigments (bp-I, blue, and bp-II, purple, see text) (from Saito, 1998) and lutein (from Jouni and Wells, 1996) indicate that the wing membrane of Df1 contains only bile pigment and that the wing membrane of Df3 contains lutein as well. Df, dorsal forewing.

A slightly different situation occurs with the reflectance of the ventral patches (Fig. 8B). The diffuse scattering by the white scales of Vf1 and Vf2 dominates the reflectance and causes a high reflectance over the whole wavelength range, and the light transmitted by the white scales is partly backscattered by the blue/green wing membrane, causing the broad peak around 500 nm. Similarly, in Vf3 and Vf4 the glass scales partly reflect light, and the transmitted light is partly backscattered by the green wing membrane.

Remarkably, the reflectance spectrum of Vf4 shows oscillations reminiscent of multilayer interference spectra (Fig. 8B, Vf4). If the glass scales indeed act as multilayer reflectors, their reflectance then will strongly depend on the angle of illumination. To investigate this we have performed imaging scatterometry (Fig. 9). A small, square, wing piece was cut out of the forewing and glued to a micropipette, which subsequently was mounted in the imaging scatterometer. A small area, diameter about 160 μm, was illuminated from various directions with white light, either from the dorsal side (Df4, Fig. 9A,C,E) or from the ventral side (Vf4, Fig. 9D,F). The hemispherical distributions of scattered light, due to illumination from about normal (Fig. 9C,D) and obliquely, with an angle of incidence of 70° (Fig. 9E,F), are shown as polar plots. The red circles correspond to the inclination angles 5°, 30°, 60° and 90°, also shown in the diagram of Fig. 9B. With about normal illumination, the light scattered by Df4 is green and quite diffuse (Fig. 9C). At very oblique illumination a slightly brighter spot is seen in the fourth quadrant, at an azimuth angle of about 280°, which is due to reflection at the wing membrane surface (Fig. 9E). Normal illumination of Vf4 results in a somewhat distributed, central light pattern, together with a diffuse green background. Oblique illumination of Vf4 results in a very bright reflection at around the 280° azimuth, clearly due to reflections from the glass scales (Fig. 9F).

The directional and strongly angle-dependent reflection of the ventral glass scales suggests the question whether or not the wing reflections may play a role in visual signaling by the butterflies during flight. The angle-dependent reflectance of interfaces can be strongly polarization-dependent, and therefore we measured the wing reflectance as a function of the angle of incidence for light polarized perpendicular (TE – transverse electric; Fig. 10A,B) and parallel (TM – transverse magnetic; Fig. 10C,D) to the plane of incidence. The illumination and measuring fibers were simultaneously varied in steps of 10°, so that the angle of the illumination fiber and that of the measuring fiber (set at the angle of reflection) were always the same. For the dorsal wing (Df4,

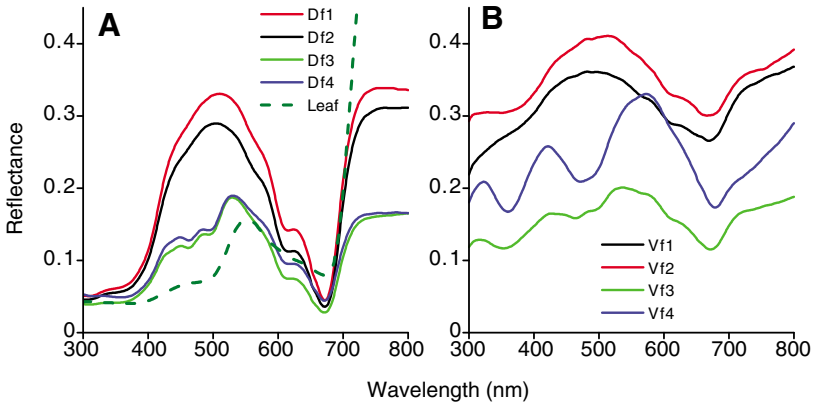

Fig. 8. Reflectance spectra of dorsal forewing patches Df1–4 (A) and ventral forewing patches Vf1–4 (B) measured with a bifurcated fiber-optic probe. For comparison, the reflectance spectrum of an oak leaf is added.

Fig. 10A,C), the TE reflectance gradually but only slightly increased with the angle of incidence at all wavelengths, and the TM reflectance only slightly increased with the angle of incidence in the long-wavelength range. Much stronger effects occurred for the ventral wing (Vf4, Fig. 10B,D). The TE reflectance increased severalfold and showed spectral peaks that moved with the angle of incidence, characteristic for thin film and multilayer reflections (the rise of the reflectance to above 1 is due to the directionality of the reflected light and the usage of a white diffuser as reference). The TM reflectance in the visible wavelength range first diminished with increasing angle of incidence, but at large angles of incidence the reflectance increased. In the long-wavelength range the TM reflectance steadily increased. The different dependencies of the reflectance spectra on angle and polarization are undoubtedly due to the glass scales on the ventral side of the wing. Possible biological functions are discussed below.

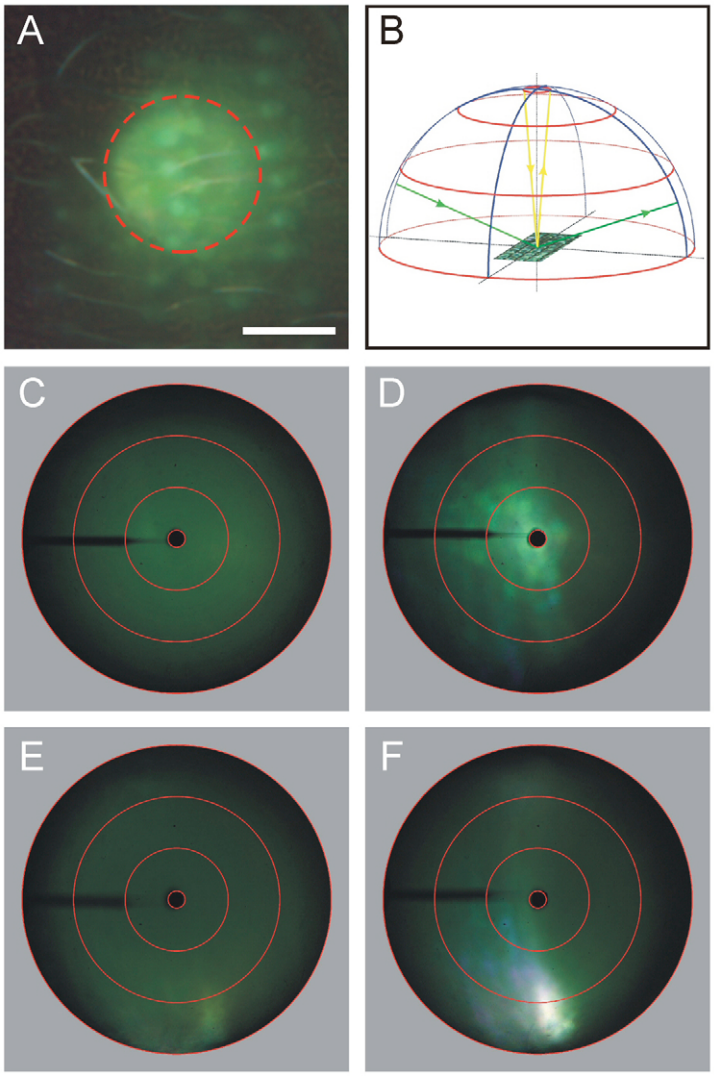

Fig. 9. Imaging scatterometry of a dorsal forewing patch Df4 (A,C,E) and its opposite side, a ventral forewing patch Vf4 (D,F). An area of about 160 μm (A; bar 100 μm), photographed through the small, central hole in the elliptical mirror of the imaging scatterometer, is illuminated with white light from the secondary beam of the scatterometer (see Stavenga et al., 2009). The explanatory diagram (B) shows how light rays with an angle of incidence 5° (yellow) or 70° (green) will leave a plane surface with the same angle (indicated by the yellow and green arrows), but generally the incident light will be scattered in various directions; the red circles indicate inclination angles of 5°, 30°, 60° and 90° The angle-dependence of the scattered light with about normal illumination (C,D) and with an angle of incidence 70° is presented in polar plots (C–F; same exposure times). The horizontal shadow in C–F is due to the glass micropipette that holds the wing piece. The scattering profile of the dorsal patch is approximately diffuse (C), but the ventral patch has a clear, specular component (D). With oblique illumination the dorsal scattering is still approximately diffuse (E), but ventrally a dominant specular reflection is seen (F).

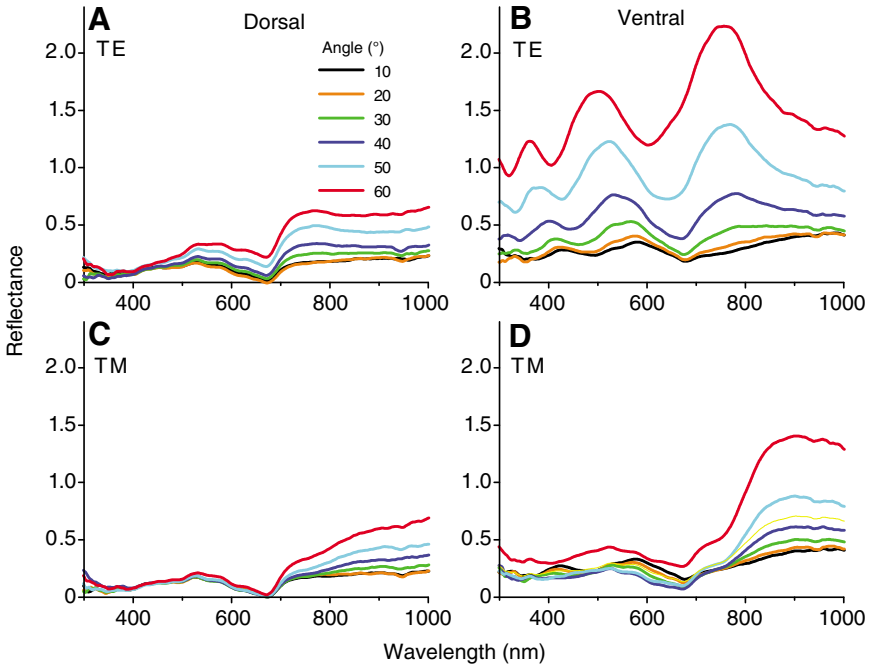

Fig. 10. Polarization and angle-dependent reflectance spectra of a dorsal forewing patch Df4 (A,C) and ventral forewing patch Vf4 (B,D), measured with two optical fibers rotating in the same plane (see Materials and methods). The illumination was with linearly polarized light, polarized perpendicular (TE, transverse electric: A,B) and parallel (TM, transverse magnetic: C,D) to the plane of incidence. The wavelength-dependent TE reflectance of the ventral side increases with the angle of incidence and exhibits peak shifts to shorter wavelengths with increasing angles of incidence.

DISCUSSION

The bile pigment sarpedobilin, locally together with the carotenoid lutein, colors the central band of wing patches of the Common Bluebottle, *Graphium sarpedon*. These pigments exist in the wing membrane and not in the wing scales, a rare case among butterflies. Actually, the bristles in the colored patches at the dorsal wing side also contain the wing membrane pigments, as can be observed with transmission, reflection (Fig. 3), as well as fluorescence light microscopy; however, the pigmentation contributes minimally to the coloration because of the bristle slenderness. The scales at the ventral side of the wings are unpigmented (Allyn et al., 1982). They are either white (in Vf1 and Vf2), because of diffuse scattering at the scale structures that surround the irregularly shaped, large windows, or they are glass-like reflective, due to the absence of windows (in Vf3–9). We have to note here that the dorsal hindwing patch Dh1 also has white, unpigmented scales (Fig. 1A). The very matte appearance of the other dorsal wing patches, which exhibit virtual no specular reflection (Fig. 9C,E), probably originates from the presence of minute lens-like structures or papillae on the dorsal surface of the wing membrane in the blue/green patches (Allyn et al., 1982).

The colored patches are surrounded by wing areas densely studded with black scales, containing melanin pigment. Removing the black scales shows that the color pigments only occur in the wing membrane of the blue/green patches. The optical and structural properties of wing membrane cells and scales and/or bristles thus are governed by connected expression systems. The wing membrane in the areas with black scales is at most very lightly brown pigmented, i.e. the membrane is very transparent throughout the whole visible wavelength range, but when covered with black scales, these wing areas have a very low transmittance, in stark contrast with the colored patches, which have a high transmittance in the blue/green, an unusual feature for butterfly wings [for other examples, see Yoshida et al. (Yoshida et al., 1997) and Yoshioka and Kinoshita (Yoshioka and Kinoshita, 2006)]. Usually layers of melanin pigment behind structurally and/or pigmentary colored scales serve to enhance the color. In the absence of a black backing, color contrast diminishes due to broad-band scattered light from the underlying wing membrane and the scales on the opposite side of the wing. This will especially have detrimental effects when the two sides of the wings are differently colored. Yet, the case of the Common Jezabel, *Delias nigrina* (Pieridae) shows that minor contributions to the color of one wing side due to backscattering from the opposite side have a negligible effect (Stavenga et al., 2006).

We have spectrophotometrically identified the two pigments of the colored patches reported before: the bile pigment sarpedobilin (Choussy and Barbier, 1973) and the carotenoid lutein (Rothschild and Mummery, 1985). Bile pigments generally occur in butterflies, notably in their larval state, but also in the wings of the adults (Rüdiger, 1970; Barbier, 1981), and carotenoids are also widespread (Rothschild and Mummery, 1985). The Green Banded Swallowtail, *Papilio phorcas*, uses the bile protein phorcabilin, which is expressed in the wing scales (Choussy et al., 1973; Barbier, 1981). Another bile pigment is pterobilin, which occurs in the wings of other *Graphium* species (e.g. *Graphium antiphates*, *Graphium agamemnon* and *Graphium doson*) and also in some nymphalids, e.g. *Nessaea* (Choussy and Barbier, 1973). Light transforms pterobilin, by cyclization, into phorcabilin, which in turn converts into sarpedobilin (Barbier, 1981). Choussy and Barbier reported that *G. sarpedon* wings contain, in addition to its main pigment, sarpedobilin, trace amounts of phorcabilin and pterobilin (Choussy and Barbier, 1973). If the latter pigments are negligible, the absorbance spectrum of the dorsal forewing patch Df1 (Fig. 7B) then is presumably representative for sarpedobilin. At least, the spectra of the bile pigments of silkworm larvae, bp-I, a phorcabilin-like pigment, and bp-II, which contains biliverdin IXγ [Fig. 7C, taken from Saito (Saito, 1998)], resemble the obtained sarpedobilin spectrum.

Fig. 7B indicates that sarpedobilin absorbs strongly in the short-wavelength as well as long-wavelength range and has little absorption in the middle-wavelength range. Accordingly, when an inhomogeneous, scattering medium contains only sarpedobilin, a blue/green color results, which is the case of wing patches Df1, Df2

and the rim of the other wing patches (Fig. 2). With lutein also present, the color changes into green, the case of wing patches Df3–8 in *G. s. nipponum* [Df9 has little lutein (Allyn et al., 1982; Rothschild and Mummery, 1985)]. The lutein content of some other subspecies, for instance *G. s. choredon* (Braby, 2000), is probably minor, as suggested by a very similar blue/green color of all wing patches. The blue-absorbing lutein causes a reflectance approximating that of leaves (Fig. 8A). The function of this yellow filter is therefore most likely to improve camouflage (Rüdiger, 1970; Kayser, 1985; Saito, 1998). Vane-Wright described five *Nessaea* species that have blue patches in the dorsal wings, due to pterobilin, but the ventral wings are mainly green (Vane-Wright, 1979). The latter is presumably due to the additional presence of a carotenoid pigment, as in some specimens, where the pterobilin is apparently lacking, the ventral wings are bright yellow. In preliminary spectrophotometrical measurements on green/yellow wing areas of *G. antiphates* and *G. agamemnon* we have found that these *Graphium* species also contain lutein.

Pieridae commonly employ bile pigments and carotenoids as larvae, but they have also been demonstrated in the adult wings (Choussy and Barbier, 1973; Feltwell and Rothschild, 1974). The wing colors of pierid butterflies are however generally determined by pterins, pigments that act as long-pass filters, resulting in either white, yellow, orange and red wing scales (Descimon, 1975; Wijnen et al., 2007). Interestingly, Feltwell and Rothschild (Feltwell and Rothschild, 1974) reported that an autosomal recessive aberration of the Large White, *Pieris brassicae ab. coerulea*, had bluish wings with transparent white scales and a green/blue pigment concentrated in the sockets, strikingly similar to the present case of *G. sarpedon*.

Although the general presence of bile pigments and carotenoids in both insect larvae and adult wings might have allowed a universal, simple coloring method, many butterfly species have green wings that are realized in different ways. Alternative tools possibly allow better matching of the wing and leaf reflectance spectra, as e.g. with the sculpted multilayers of *P. palinurus* scales (Vukusic et al., 2001), the overlapping curved wing scales of *C. rhipheus* (Yoshioka and Kinoshita, 2007) or the photonic crystal gyroids of the ventral wing scales of *C. rubi* (Michielsen and Stavenga, 2008; Stavenga et al., 2009; Michielsen et al., 2010). But perhaps a much more important aspect of these wave-optics-governed cases is that the reflectance spectra depend on the angle of incidence and polarization. Butterflies, like most other insects, have polarization vision (Rossel, 1989; Bandai et al., 1992; Kelber et al., 2001), but this modality is presumably absent in their main predators, birds (Greenwood et al., 2003). The polarized iridescence of butterfly wings may play a crucial role in intraspecific signaling. For instance, the nymphalid Cydno Longwing, *Heliconius cydno*, may use polarized light as a private communication channel, minimizing detection by predators while maximizing conspicuousness to potential mates (Sweeney et al., 2003; Douglas et al., 2007). Similarly, the glass scales on the ventral wings of *G. sarpedon* exhibit a distinct polarized iridescence (Fig. 10B,D). The polarized iridescence of the ventral wings may therefore play a role in intraspecific communication.

The additional optical function of the glass scales is to contribute to the dorsal coloration. This is achieved in two ways: (1) the glass scales partly reflect incident light from the dorsal side that is transmitted by the wing, and, by having a second chance to be absorbed, the doubly transmitted light fraction enhances the green hue; and (2) the largely transparent glass scales allow light incident from the ventral side to be green-filtered by the pigments in the wing and so further enhance the green hue. We conclude that in highly transparent wing areas, the case of the blue/green pigmented patches of *G. sarpedon*, the resulting color depends on incident illumination from both sides of the wing.

LIST OF ABBREVIATIONS

ARM	angle-dependent reflectance measurements
Df	dorsal forewing
ISM	imaging scatterometer
MSP	microspectrophotometer
SEM	scanning electron microscopy
TE	transverse electric
TM	transverse magnetic
Vf	ventral forewing
Vh	ventral hindwing

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